Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Professor Lian-Sheng Ma
Editor-in-Chief
World Journal of Gastrointestinal Oncology

14th of February, 2021

Dear Professor Ma

Name of Journal: *World Journal of Gastrointestinal Oncology*
Manuscript NO. : 62058, Review

The authors would like to thank the reviewer for their helpful comments and time taken to review this manuscript. We have edited the manuscript to improve general readability and clarity, addressed the reviewer, and followed the directions from the science editor below.

Reviewer #1:
Hypoxia is a common phenomenon among various solid tumors. This review discussed the important role of hypoxia microenvironment in tumor angiogenesis, invasion, metastasis and treatment resistance, as well as the new progress of hypoxia targeted therapies. It is an excellent work. I think the titles and contents of the section of introduction should be revised.

We agree with the reviewer and thank you for this suggestion. As part of the revision we have introduced the concept of hypoxia earlier, trimmed some of the content to avoid repetition and removed the subsections to the introduction. We hope this improves the readability of the manuscript. Please see the new introduction below.

" One of the major turning points in the study of solid tumours arose with the realisation that a critical regulatory influence in the process of angiogenesis was an environmental feature; hypoxia [1,2]. Many studies have since demonstrated the oncogenic transforming power of hypoxia in the microenvironment of different tumour types and the observation that tumour oxygenation status could disrupt the anti-tumour effects of radiation therapy was published over 60 years ago [3–8]. This review will discuss the role of hypoxia in the tumour microenvironment (TME) of gastroesophageal cancers (GOCs) including gastric cancer (GC) and oesophageal cancer (OC), how it augments disease, and additionally its relevance in the setting of prognostication and therapeutic targeting.

Roinn na Máinliachta
Ionad Choláiste na Tríonóide do na hEolaíochtaí Sláinte
Ospidéal San Séamas,
Baile Átha Cliath 8, Éire

Department of Surgery
Trinity Centre for Health Sciences
St. James's Hospital,
Dublin 8, Ireland

T + 353 (0)1 896 2189
F + 353 (0)1 896 4122

surgery@tcd.ie
www.medicine.tcd.ie/Surgery

GOC is a substantial cause of morbidity and mortality, responsible for 1.2 million deaths per year globally [9–12]. An improved understanding of the risk factors for GC has seen a steady decline in both the incidence and mortality which is in sharp contrast to the rising incidence of OC, particularly oesophageal adenocarcinoma (OAC) globally [13,14]. GOCs develop insidiously and consequently, are commonly diagnosed at an advanced stage where chemotherapy with or without radiation remains the treatment of choice in the neoadjuvant setting [15]. Treatment at this stage is rarely curative and several mechanisms account for this resistance to treatment including tumour cell-intrinsic and extrinsic mechanisms. Hypoxia is a characteristic feature of the TME and a key mediator in conferring and enhancing treatment resistance [16–18]. The TME being the complex reciprocity between both the cellular (resident and infiltrating) and non-cellular components that surround, envelop and make up the tumour mass, the components of which are summarised in **Figure 1** [19–21]. The exact mechanisms underlying resistance continue to be elucidated and as such, interest in the role of hypoxia in translational oncology research has garnered increasing interest in recent history as shown in **Figure 2.**

Hypoxia mediates aggressive, metastatic, and treatment-resistant disease by augmenting the hallmarks of cancer through various cellular and physiological events including; enhanced tumour cell proliferation, survival, immune evasion, inflammation, induction of angiogenesis, and activation of invasion [16,17,22]. In large part these events are influenced or orchestrated by the relationship between oxygen availability and the genes encoding Hypoxia-Inducible Factors (HIF) and Von Hippel Lindau protein (pVHL) [23,24]. HIFs are a family of heterodimeric transcription factors consisting of a labile α subunit and a stable β subunit. There are several HIF isotypes but the most well-studied is HIF1. HIF1-α contains domains amenable to post-translational modifications thereby mediating interactions with the molecular machinery responsible for cellular degradation [25,26]. When induced, HIF1-α associates with the constitutively expressed HIF1-β and together act to bring about the transcription of a multitude of genes involved in complex signalling pathways with a diverse degree of roles. There exists a whole host of HIF target genes that are transcribed in response to hypoxia that have been implicated in driving tumour progression. The roles of these target genes range from receptors to enzymes to further transcription factors and more (**Table 1**), which are involved in the enhancement of inflammation, angiogenesis, immune evasion, and the other remaining hallmarks of cancer.

In the setting of normoxia, HIF1-α is regulated by two principal mechanisms; oxygen-dependent pVHL-dependent degradation, and oxygen-dependent non-pVHL-dependent inactivation (**Figure 3**)[25,27,28]. Hydroxylation by oxygen-dependent prolyl hydroxylase domain enzymes (PHDs) triggers recognition by the E3 ubiquitin ligase, pVHL, ensuring proteasomal degradation. In the non-pVHL dependent pathway, induction of Factor Inhibiting HIF (FIH) leads to hydroxylation of an asparagine residue

preventing HIF1-α from localising with the co-activators p300 and CBP, hence disabling transcriptional activation [29].

The contribution of hypoxia to disease progression makes it an attractive therapeutic target and potential prognostic aide. However, in the setting of GOC, there are currently no agents specifically targeting hypoxia, nor are there any biomarkers that assess the extent of tumour hypoxia, to guide treatment choice or to indicate the likelihood of treatment response. In this era of precision medicine, a validated biomarker would improve the standard of care for this group of patients."

Science editor:

1. I found no "Author contribution" section. Please provide the author contributions.

   Thank you for pointing this out, the below has been added to the revised manuscript.

   "**Author contributions:** King R wrote the manuscript; King R, Hayes C, Donohoe CL, Dunne M, Davern M, and Donlon NE conceived the work and made substantial revisions to and critique of the content. All authors have read and approve the final manuscript."

2. I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

   Thank you for the comment. The original figures have been prepared and arranged in the PowerPoint file titled "62058-Figures.ppt". Figure 2 is an original figure designed using R. This has been added to the caption. Figure 1 and 4 are original figures created by BioRender.com and cannot be further decomposed outside their proprietary application. Figure 3 was adapted from a template available by BioRender.com. These captions have been updated to reflect this. If the editor has trouble with formatting the figures we would be happy to adjust them.

   "**Figure 1. The components of the TME are affected by hypoxia in numerous ways.** Important cellular components of the TME include immune cells like macrophages, dendritic cells, myeloid-derived suppressor cells, T cells, natural killer (NK) cells, as well as cancer-associated fibroblasts. Non-cellular aspects include the extracellular matrix and signalling molecules like VEGF, adenosine, and cytokines and chemokines including IL-6, IFN-γ, CXCL1, CXCL3, CCL28 [12–14,40]. CAF=cancer associated fibroblasts. ECM=extracellullar matrix. EMT=epithelial-mesenchymal transition. HIF=hypoxia inducible factor. NFκB=nuclear Factor-kappa light chain enhancer of activated B cells.

OxPhos=oxidative phosphorylation. ROS=reactive oxygen species VEGF=vascular endothelial growth factor. Created with BioRender.com"

"**Figure 2. The amount of research investigating the role of hypoxia in cancer has increased over the past 20 years as seen as a proportion of PubMed listed articles.** Created using R: A Language and Environment for Statistical Computing"

"**Figure 3. Regulation of HIF1-α by oxygen levels and pVHL.** Hydroxylation by oxygen-dependent prolyl hydroxylase domain enzymes (PHDs) triggers recognition by the E3 ubiquitin ligase VHL, ensuring proteasomal degradation. In the non-pVHL dependent pathway, induction of Factor Inhibiting HIF (FIH) leads to hydroxylation of an asparagine residue preventing HIF1-α from localising with the co-activators p300 and CBP, hence disabling transcriptional activation [30]. The HIF pathway functions to conduct and orchestrate the cellular response to low oxygen availability [24,25]. HRE = Hypoxia response element. ARNT = Aryl hydrocarbon receptor nuclear translocator. Adapted from "HIF Signalling", by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates"

"**Figure 4. The effects of hypoxia on immune evasion.** Hypoxia has been shown to impair antigen uptake and migration in dendritic cells while at the same time increasing VEGF production thus impairing the bridge between the innate anticancer immune response and the adaptive response while also enhancing angiogenic signalling. HIF-mediated transcription of the cytokine IL-6 and FoxP3 results in the subsequent recruitment of immunosuppressive MDSCs and in increased proportion of protumourigenic Tregs respectively. Low oxygen status is also linked with decreased tumour expression of the NK cell receptor ligand MICA, as well as its receptor NKG2D on NK cells. Hypoxia-dependent transcription of chemokines such as CCL2 and CCL5 enhance the recruitment of protumour TAMs through receptors such as CXCR4. DC=dendritic cell. MDSC=myeloid derived suppressor cell. NK cell=natural killer cell. TAM=tumour associated macrophage. Treg cell=T regulatory cell. VEGF=vascular endothelial growth factor. Created with BioRender.com"

The original tables have also been prepared and arranged in the Word document titled "62058-Tables.docx"

3. I found the authors did not add the PMID and DOI in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list, and list all authors of the references. Please revise throughout.

Thank you for pointing this out. The Article Reference Tool and Auto-Analyser were used to add missing PMIDs/ DOIs where required. References 2, 3, 8, 32, 42, 112, 121, 133, 158, and 177 do not have a DOI. Reference 121 and 177 also do not have a PMID. These two are highlighted in yellow as per the Guidelines and Requirements for Manuscript Revision Review

4. Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere , or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly indicating the reference source and copyrights. For example, "Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]". And please cite the reference source in the references list. If the author fails to properly cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to withdrawal of the article from BPG publications and may even be held liable.

   Thank you for drawing our attention to this, we believe our response in point number 2 above, should clarify this. For more information, please see the following webpage.

   How do I cite BioRender? | BioRender Help Center

We would once again like to thank you for considering our manuscript for publication in the *World Journal of Gastrointestinal Oncology*.

Kind regards,

Noel E Donlon, MB BCh BAO MRCS